

Ann Isabel SANZ MOLINERO
Appl. No. 10/537,897
Atty. Ref.: 4982-5
Amendment
Monday, March 31, 2008

AMENDMENTS TO THE SPECIFICATION:

Please amend the specification as follows:

Please delete the paragraph spanning lines 4-22 of page 10 and insert the following therefor:

Methods for the search and identification of 2xC2H2 zinc finger protein homologues, for example STZ zinc finger homologues, would be well within the realm of a person skilled in the art. Such methods, involve screening sequence databases with the sequences provided by the present invention, for example SEQ ID NO 2 (or SEQ ID NO 1), preferably in a computer readable format. This sequence information may be available in public databases, that include but are not limited to Genbank (URL: ncbi.nlm.nih.gov/web/Genbank) (<http://www.ncbi.nlm.nih.gov/web/Genbank>), the European Molecular Biology Laboratory Nucleic acid Database (EMBL) (URL: w.ebi.ac.uk/ebi-docs/embl-db.html) (<http://w.ebi.ac.uk/ebi-docs/embl-db.html>) or versions thereof or the MIPS database (URL: mips.gsf.de/) (<http://mips.gsf.de/>). Different search algorithms and software for the alignment and comparison of sequences are well known in the art. Such methods include GAP, BESTFIT, BLAST, FASTA and TFASTA. GAP uses the algorithm of Needleman and Wunsch (J. Mol. Biol. 48: 443-453, 1970) to find the alignment of two complete sequences that maximises the number of matches and minimises the number of gaps. The BLAST algorithm calculates percent sequence identity and performs a statistical analysis of the similarity between the two sequences. The suite of programs referred to as BLAST programs has 5 different implementations: three designed for nucleotide sequence queries (BLASTN, BLASTX, and TBLASTX)

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and two designed for protein sequence queries (BLASTP and TBLASTN) (Coulson, Trends in Biotechnology: 76-80, 1994; Birren et al., GenomeAnalysis, 1: 543, 1997). The software for performing BLAST analysis is publicly available through the National Centre for Biotechnology Information.

Please delete the paragraphs spanning lines 1-19 on page 11 and insert the following therefor:

The identification of such domains or motifs for examples the motif and boxes as represented by SEQ ID NO 5, 6, 7, 8 and 9, would also be well within the realm of a person skilled in the art and involves for example, a computer readable format of proteins of the present invention, the use of alignment software programs and the use of publicly available information on protein domains, conserved motifs and boxes. This protein domain information is available in the PRODOM (URL: biochem.ucl.ac.uk/bsm/dbbrowser/jj/prodomsrchjj.html) (<http://www.biochem.ucl.ac.uk/bsm/dbbrowser/jj/prodomsrchjj.html>), PIR (URL: pir.georgetown.edu/) (<http://pir.georgetown.edu/>) or pFAM (URL: pfam.wustl.edu/) (<http://pfam.wustl.edu/>) database. For the identification of Zinc finger domains, such as the 2xC2H2 zinc finger domain, pFAM is preferred. Sequence analysis programs designed for motif searching may be used for identification of fragments, regions and conserved domains as mentioned above. Preferred computer programs would include but are not limited to MEME, SIGNALSCAN, and GENESCAN. A MEME algorithm (Version 3.0) may be found in the GCG package; or on the Internet site URL:

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sdsc.edu/MEME/meme <http://www.sdsc.edu/MEME/meme>. SIGNALSCAN version 4.0

information is available on the Internet site URL:

biosci.cbs.umn.edu/software/sigscan.html

<http://biosci.cbs.umn.edu/software/sigscan.html>. GENESCAN may be found on the Internet site URL: gnomic.stanford.edu/GENESCANW.html

<http://gnomic.stanford.edu/GENESCANW.html>.

At present, zinc finger motifs are subdivided in more than 40 different classes as can be found in the Pfam database of protein families present at the Sanger institute (URL: sanger.ac.uk/Software/Pfam/browse/Z.shtml) (<http://www.sanger.ac.uk/Software/Pfam/browse/Z.shtml>).

Please delete the paragraph spanning lines 18-30 on page 14 and insert the following therefor:

Othologues in other plant species may easily be found by performing a so-called reciprocal blast search. Orthologous genes can be identified by querying one or more gene databases with a query gene or protein of interest (SEQ ID NO 1 or 2), using for example BLAST program. The highest-ranking subject genes that result from the search are then again subjected to a BLAST analysis, and only those subject genes that match again with the query sequence (SEQ ID NO 1 or 2) are retained as true orthologous genes. For example, to find a rice orthologue of an *Arabidopsis thaliana* gene, one may perform a BLASTN or TBLASTX analysis on a rice database such as (but not limited to) the *Oryza sativa Nipponbare* database available at the NCBI website

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(URL: ncbi.nlm.nih.gov) (<http://www.ncbi.nlm.nih.gov>) or the genomic sequences of rice (cultivars *indica* or *japonica*). In a next step, the obtained rice sequences are used in a reverse BLAST analysis using an *Arabidopsis* database. The results may be further refined when the resulting sequences are analysed with ClustalW and visualised in a neighbour joining tree. The method can be used to identify orthologues from many different species.

Please delete the paragraph spanning lines 11-17 on page 16 and insert the following therefor:

A phylogenetic tree may be constructed with all the homologues, paralogues and orthologues are defined herein above. Multiple alignment may be made using clustal W present in the VNTi (version 5.0) program with for example Gap opening penalty 10 and Gap extention 5. For making a phylogenetic tree the Phylic software package available at URL: evolution.genetics.washington.edu/phylip.html <http://evolution.genetics.washington.edu/phylip.html> may be used. Sequences clustering around SEQ ID NO 1 or SEQ ID NO 2, identify genes or proteins suitable for use in the methods of the present invention.

Delete Table I (a), spanning lines 11-13 on page 22 and insert the following therefor:

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Gene	Expression	Reference
AtPRP4	flowers	<u>URL:</u> salus.medium.edu/mmg/tierney/html http://salus.medium.edu/mmg/tierney/html
chalcone synthase (chsA)	flowers	Van der Meer, <i>et al.</i> , <i>Plant Mol. Biol.</i> 15, 95-109, 1990.
LAT52	anther	Twell <i>et al</i> <i>Mol. Gen Genet.</i> 217:240-245 (1989)
<i>apetala-3</i>	flowers	

Please insert the following new paragraph on line 9 of page 34:

BRIEF DESCRIPTION OF THE DRAWINGS